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MATE GUARDING IN AMPHIPODS: A ROLE FOR BROOD POUCH STIMULI

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Abstract

In the amphipod, *Gammarus lawrencianus,* females carry fertilized eggs and occasionally brood juveniles in a ventral brood pouch. The male's decision to adopt and guard a female for mating purposes is influenced by the contents of the brood pouch. The presence of juveniles or recently fertilized eggs in the brood pouch inhibits the mate guarding decision; and an empty brood pouch facilitates it.

Introduction

In many animals, the male and female join together for a brief period of several days prior to mating. This phenomenon is called precopulatory mate guarding with reference to the observation that the male typically defends the female from other males during this period. This reproductive strategy has been of particular interest to sociobiologists and population biologists because of its implications for theories of sexual selection, and its potential contribution to our understanding of mating systems in general (Trivers, 1972; Parker, 1974; Wickler and Seibt, 1979; Grafen and Ridley, 1983).

Among invertebrates, precopulatory mate guarding is widespread in the major groups of Crustacea (Dunham, 1978; Atema and Cobb, 1980; Salmon, 1983). *Gammarus,* for example, is a large genus of amphipods that have adapted to almost every type of aquatic habitat in the world. In spite of this diversity, the reproductive strategies of these small crustaceans are surprisingly uniform. *Gammarus lawrencianus,* a euryhaline amphipod which is found in estuaries along the northeast coast of North America is a typical example. In this polygynous species, mating occurs immediately after each female molt. Following sperm transfer, the female releases eggs into a specialized ventral brood pouch where they are fertilized. The embryos develop in the brood pouch for a number of days and eventually hatch as juveniles that are released into the water column. This cycle is typically repeated every 9–12 days during the reproductive season.

Precopulatory mate guarding can be initiated at any time prior to the female molt. A highly stereotyped sequence of behavior is elicited when a free-swimming male comes into contact with an appropriate female. The male grabs her with his gnathapods and draws her close to his ventral surface in a position which places the longitudinal axes of their bodies at right angles to each other. In this position he actively palpates the female with various appendages including his antennules. After a period of palpation, the female is either rejected *or* rotated into the precopula position with the female underneath the body of the male and their body axes parallel. The pair swims together in this precopula position until the female molts and sperm transfer is com-

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pleted, at which point they separate. Available evidence suggests that the females adopt a passive role throughout this process, and various laboratory and field observations indicate that the probability of finding a female in precopula with a male increases as she approaches the final stages of her molt cycle (Hartnoll and Smith, 1978; Birkhead and Clarkson, 1980; Hunte *et al.,* 1985; Dunham *et al.,* in press).

Experiment 1

Over the past few decades a considerable amount of research and speculation has attempted to identify a critical stimulus (or stimuli) which elicits precopulatory mate guarding in amphipods (Dunham, 1978, in press). The most viable hypothesis to date is that a pheromone released by the female mediates this process. However, several different investigators have questioned this assumption, (*cf.* Dunham, 1978; Hartnoll and Smith, 1980; Borowsky, 1984), and, at present, the specific factors which control the mate guarding decision have not yet been isolated.

Recently, some personal, informal observations of *G. lawrencianus* in a breeding culture suggested that the contents of the female's thoracic brood pouch might play a role in the male's guarding decision. First, during the palpation phase, the females are held in a position which permits the male to palpate this structure; second, the female is accepted or rejected after this palpation; and finally, close inspection of various females suggested that females with an empty brood pouch are accepted more quickly than those carrying juveniles. This first experiment was designed to provide a more formal test of these informal observations by directly comparing females with an empty brood pouch to those carrying juveniles to determine if there are behavioral differences in their mate guarding interactions.

Materials and methods

Subjects. A semi-naturalistic 20 l breeding culture of *G. lawrencianus* maintained at a field station on St. Margaret's Bay, Halifax County, Nova Scotia, was the source of all animals used in these experiments. Small stones and fucoids provided natural cover in the culture, and the animals were exposed to the natural day-night cycle occurring at this latitude from January to March. Water temperature varied from a low of 8°C at night to a daytime high of 18°C. Animals were demand-fed Tetra Marin fish food, small bits of mussel (*Mytilus edulis*), and dried squash flakes. Under these conditions, the average length of the female reproductive cycle (copulation to copulation) was 14.8 days with, on average, the last 5 days of each cycle spent in precopula with a male.

Procedure. Twenty-eight precopula male-female pairs were sampled from the breeding culture for observation. All observations were conducted between 1200 and 1400 hours at a water temperature of 15 ± 2°C. Prior to the observations each precopula pair was gently separated and placed in individual 50 ml containers of seawater. Following this brief period of isolation, both containers were emptied into a common third container (6 cm diameter) that served as a test arena where the animals could re-establish the mate guarding relationship. An observer maintained a real-time record of the number of contacts between the animals and the category and duration of each contact. Contacts were coded into two different categories: (a) contacts in which the male positioned the female for *palpation;* and (b) contacts in which the female was placed in the *precopula* position. Following each test, the female was examined under a microscope to determine the state of her brood pouch.

Results

Of the 28 pairs sampled from the breeding culture, 14 females had empty brood pouches, 12 carried recently hatched juveniles, and 2 pairs, damaged during separation, were excluded from the analysis.

The behavioral differences between these two different groups of females during the mate guarding assay are presented in Table I. These data make three important points. First, males take approximately the same amount of time to establish an initial palpation contact with either type of female (juveniles present *vs.* juveniles absent). Second, once a palpation contact is made, the time required to make the precopula decision is significantly shorter with females that have empty brood pouches ($t = 3.9$, $P < .005$). Third, mate guarding was eventually re-established in all pairs regardless of the brood pouch contents. Apparently, the presence of juveniles in the brood pouch does not alter the initial "attractiveness" of the female or prevent eventual mate guarding, but the juveniles do increase the time it takes the male to decide to guard the female.

Experiment 2

Changes in the contents of the female's brood pouch would be an adaptive stimulus upon which to base the precopulatory mate guarding decision. The presence or absence of embryos and the state of their development provides easily discriminated information about the time remaining before the next opportunity to copulate. Large, yolk-filled, recently fertilized eggs predict a long mate guarding investment, whereas an empty brood pouch signals a short investment time. However, there are other changes in the female which are perfectly correlated with changes in the brood pouch contents, and are also able to predict the time remaining until copulation. For example, changes in both molt physiology and ovary development are correlated with embryonic development in the brood pouch. Although the data from Experiment 1 suggest that brood pouch contents influence the mate guarding decision process, these other confounded changes may also be necessary and/or sufficient.

The strongest possible test of the hypothesis that brood pouch contents are responsible for the differences in behavior observed in Experiment 1 would pit the brood pouch stimuli against all other changes in the female's reproductive and molt physiology and determine whether the male bases his mate guarding behavior on the brood pouch contents, or on these other factors normally correlated with changes in the brood pouch contents. The next experiment was designed to perform this test.

Female *G. lawrencianus* are seldom guarded immediately after sperm transfer when the brood pouch is full of large recently fertilized, yolk-filled eggs which, according

Table I

The average time in seconds to the first palpation response by the male; the average time from the first palpation response to the precopula position, and the percentage of pairs that were in the precopula mate guarding position when the three minute test session was terminated

	Juveniles present in brood pouch	No juveniles in brood pouch
Average time to first palpation response	51.3 s	52.2 s
Average time from palpation response to precopula	80.1 s	25.3 s
Percentage mate guarding at end of test	100%	100%

to criteria described by Steele and Steele (1969), are Stage A embryos. Only two percent of the precopula mate guarding pairs observed in various samples from this breeding culture involve females carrying Stage A embryos. According to the reasoning outlined above, it should be possible to transform these unattractive, unguarded females into attractive, guarded females by aborting their embryos. An aborted female presents the male with a brood pouch which signals a short investment time, while all other aspects of her reproductive and molt physiology signal a long investment time.

Materials and methods

All maintenance conditions and test conditions used in the present experiment were identical to those described in Experiment 1, with exceptions noted below. For the present experiment, 30 unguarded females carrying Stage A embryos in their brood pouch were sampled from the breeding culture. The embryos were flushed from the brood pouch of 15 of these females (without apparent damage to the animals) by spraying a stream of seawater from a small glass capillary tube into the pouch. Another 15 females were exposed to a similar treatment directed at the outside of the brood pouch so that the embryos were sprayed but not removed. One hour after flushing, each female was placed in a test container with a male and observed for three minutes using the same behavioral assay described earlier. The males used in these assays were obtained from precopula pairs randomly selected from the breeding culture, separated from their female partners, and maintained in isolation for one hour prior to the behavioral assay in which they participated. A third comparison group consisted of 15 untreated, "normal" male-female precopula pairs randomly selected from the culture, separated for one hour and observed for three minutes as they re-established the precopula mate guarding position.

Results

The results of these treatments are seen in Table II. The important comparison is between the previously unguarded females carrying Stage A embryos and unguarded females at the same stage of the reproductive cycle with their embryos aborted. It is evident that a female with aborted embryos is more likely to be guarded by a male (53%) than a female at the same stage of the reproductive cycle with the same embryos

TABLE II

Average time in seconds to the first palpation response made by males; average time from palpation to the precopula position; and percentage of pairs in mate guarding position at the termination of the three minute test session

	Isolated females with embryos aborted (n = 15)	Isolated females with embryos in pouch (n = 15)	Precopula females with empty pouch (n = 15)
Average time to first palpation	30 s	35.6 s	41 s
Average time from palpation to precopula	83.3 s*	145.7 s	55.3 s
Percentage mate guarding	53%	6%	80%

* If only those males which successfully re-established precopula pairing with these females are considered, the average time from palpation to precopula was 24.6 seconds.

in her brood pouch (6%). However, aborting the embryos does not make the female as attractive as a "normal" guarded female with an empty brood pouch (80% mate guarding). ($\chi^2 = 16.55$, $P < .001$).

The second measure of interest in Table II is the time taken to make the precopulatory mate guarding decision once the male starts to palpate the female. The data reveal that the time taken from the first palpation response to the final precopulatory mate guarding response is significantly shorter for females with empty brood pouches, whether the embryos were aborted or vacated the pouch normally ($F = 8.8$, $P < .01$). It should be noted with reference to this decision time measure that the pairs which failed to achieve precopulatory mate guarding during the test period were assigned a decision time based on the interval between their first palpation response and the termination of the 3 min test session. In fact, the positive effects of the abortion procedure on decision time is even more impressive when one considers only those pairs in which precopulatory mate guarding was successfully established during the test session. The eight aborted females (53%) that were successfully guarded by males required an average decision time of only 24.4 seconds. This decision time was slightly faster than the average time required by the 12 (80%) successfully guarded "normal" females with empty brood pouches (33.7 seconds). Apparently, if the male decides to guard a female with an empty brood pouch, the decision time is short and approximately the same in response to aborted and normally empty brood pouches.

Finally, it should be noted that the amount of time required for the male to make the first palpation response did not differ in the three different groups of females ($F = <1$). Once again, brood pouch contents do not appear to have an effect on the attractiveness of the female upon initial contact.

Discussion

The evidence from these two experiments demonstrates that the contents of the female brood pouch influences the male's mate guarding decision. This appears to be an adaptive reproductive strategy. The male amphipod's decision to guard a female with an empty brood pouch minimizes time and energy expended on the female and/or a previous male's offspring, and also assures the guarding male's paternity. To the extent that the brood pouch contains juveniles (or recently fertilized eggs), the decision to mate guard represents a more substantial investment of time and energy for the same gain (Parker, 1974).

The fact that a female with an aborted brood pouch is not quite as attractive as a "normal" female with an empty brood pouch (Exp. 2) suggests that factors in addition to the brood pouch contents are also contributing to the final precopula decision. One possibility is that the female actively participates in the mate guarding interaction. Females at different stages of their reproductive cycle may be more or less cooperative with the male, and this possibility needs to be examined with assays that are sensitive to changes in both male and female behavior during the mate guarding decision process.

Finally, these data do not exclude the possibility that a pheromone is involved in the precopulatory mate guarding of *G. lawrencianus*. They do, however, suggest that the communication process is more complex than previously considered. My observations of these animals, and descriptions of other species, suggest that there are two critical points in the mate guarding decision sequence: at initial contact the male either ignores the female or palpates her; and after palpation, the male either rejects the female or places her in the precopula position. The present data indicate that a different set of stimuli mediate these two different components of the mate guarding behavior.

Initial contact decisions may be based on a water borne or contact pheromone (*cf.* Hartnoll and Smith, 1980; Borowsky, 1984) and the subsequent precopula decision may be based on tactile, visual, and/or chemical information about the contents of the brood pouch. Techniques are currently being developed to systematically insert and extract various brood pouch contents to discover the stimulus dimension(s) which control the precopula decision.

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